

FORM PTO-1390 (REV. 10-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 20267P
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <b>09/806088</b>
INTERNATIONAL APPLICATION NO. PCT/US99/23253	INTERNATIONAL FILING DATE 10/05/1999	PRIORITY DATE CLAIMED 10/09/1998
TITLE OF INVENTION DELTA 6 FATTY DESATURASE		
APPLICANT(S) FOR DO/EO/US KONSTANTIN PETRUKHIN AND C. THOMAS CASKEY		
<p><b>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</b></p> <ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This is an express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made and the US was elected by the expiration of the 19th month from the earliest claimed priority date (PCT Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed [35 U.S.C. 371(c)(2)].           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input type="checkbox"/> An English language translation of the International Application as filed [35 U.S.C. 371(c)(2)].</li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)].           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].</li> <li>10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].</li> </ol> <p><b>Items 11 to 16 below concern other document(s) or information included:</b></p> <ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input type="checkbox"/> A <b>FIRST</b> preliminary amendment.  <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A substitute specification.</li> <li>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>16. <input type="checkbox"/> Other items or information:</li> </ol>		

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DATE OF DEPOSIT March 26 2001

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DATE 3/26/01

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/806088		PCT/US99/23253		20267P	

17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]:</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee [37 CFR 1.445(a)(2)] paid to USPTO and International Search Report not prepared by the EPO or JPO..... <b>\$1,000.00</b> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... <b>\$860.00</b> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee [37 CFR 1.445(a)(2)] paid to USPTO..... <b>\$710.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... <b>\$690.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... <b>\$100.00</b> <div style="text-align: right;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div>				CALCULATIONS		PTO USE ONLY	
				\$100.00			
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(e)].				\$0.00			
Claims	Number Filed	Number Extra	Rate				
Total Claims	15 - 20 =	0	X <b>\$18.00</b>	\$0.00			
Independent Claims	3 - 3 =	0	X <b>\$80.00</b>	\$0.00			
Multiple dependent claim(s) (if applicable)			0	+ <b>\$270.00</b>	\$0.00		
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$100.00			
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.							
<b>SUBTOTAL =</b>				\$100.00			
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(f)].				\$0.00			
<b>TOTAL NATIONAL FEE =</b>				\$100.00			
Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property.				\$0.00			
<b>TOTAL FEES ENCLOSED =</b>				\$100.00			
				Amount to be refunded			
				charged			

a. ☐ A check in the amount of \$ \_\_\_\_\_ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 13-2755 in the amount of \$100.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to the Deposit Account No. 13-2755. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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 Patent Department, RY60-30  
 P.O. Box 2000  
 126 East Lincoln Avenue  
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DATE: MARCH 26, 2001

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 SIGNATURE  
 Joseph A. Coppola  
 \_\_\_\_\_  
 NAME  
 38,413  
 \_\_\_\_\_  
 REGISTRATION NUMBER

## TITLE OF THE INVENTION

DELTA 6 FATTY ACID DESATURASE

## CROSS-REFERENCE TO RELATED APPLICATIONS

5 Not applicable.

## STATEMENT REGARDING FEDERALLY-SPONSORED R&amp;D

Not applicable.

## 10 REFERENCE TO MICROFICHE APPENDIX

Not applicable.

## FIELD OF THE INVENTION

15 The present invention is directed to novel human DNA sequences encoding a delta 6 fatty acid desaturase, an enzyme involved in the synthesis of essential fatty acids.

## BACKGROUND OF THE INVENTION

20 Essential fatty acids (EFAs) are polyunsaturated fatty acids that cannot be manufactured by mammals, yet are required for a number of important biochemical processes, and thus must be supplied in the diet. The most important dietary EFAs are linoleic acid and alpha-linolenic acid (ALA). These two EFAs undergo a number of biosynthetic reactions that convert them into various other EFAs. Figure 1 depicts the biosynthetic reactions involving the two groups of EFAs, the n-6 EFAs (linoleic acid derivatives) and the n-3 EFAs (ALA derivatives). EFAs are formed from linoleic acid and ALA by a series of alternating reactions involving the removal of two  
25 hydrogens coupled with the insertion of an additional double bond (desaturation) and the lengthening of the fatty acid chain by the addition of two carbons (chain elongation). The enzymes catalyzing the desaturations and elongations are thought to  
30 be the same for both groups of EFAs.

Among the more important unsaturated fatty acids are the delta 6 unsaturated fatty acids, which are involved in the maintenance of membrane structure and function, the regulation of cholesterol synthesis and transport, and the prevention

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of water loss from the skin. Delta 6 unsaturated fatty acids also serve as precursors of the eicosanoids, including the prostaglandins and leukotrienes (Horrobin, 1992, Prog. Lipid Res. 31:163-194). The double bond at the 6 position of delta 6 unsaturated fatty acids is introduced by a class of enzymes known as delta 6 desaturases.

5 Deficiencies in linoleic acid and ALA derivatives have been associated with skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction. For example, a deficiency in gamma-linolenic acid (GLA), which is produced from linoleic acid by the action of the enzyme delta 6 desaturase, can arise from the  
10 decreased activity of this enzyme that occurs in aging, stress, diabetes, eczema, and some infections, or from increased catabolism of GLA due to oxidation or rapid cell division, as occurs in inflammation or cancer. Clinical trials have demonstrated that dietary GLA supplementation can be effective in treating a number of conditions that are associated with GLA deficiency, *e.g.*, atopic eczema, mastalgia, diabetic  
15 neuropathy, viral infections, and some forms of cancer (Horrobin, 1990, Rev. Contemp. Pharmacother. 1:1-45).

Delta 6 desaturase is an example of a fatty acid desaturase. Fatty acid desaturases are enzymes that introduce a double bond into the carbon chain of fatty acids. They play vital roles in the biosynthesis of polyunsaturated fatty acids,  
20 including the essential fatty acids. Fatty acid desaturases are present in soluble and membrane-associated forms and require electron donors (for example, cytochrome b5) for their functioning.

Delta 6 desaturases catalyze the rate-limiting steps in the biosyntheses of the linoleic and ALA group EFAs shown in Figure 1. End products of the linoleic acid pathway include the eicosanoids (prostaglandins and leukotrienes). The end  
25 product of the ALA pathway is docosahexaenoic acid (DHA), an important component of membranes in the vertebrate retina. DHA is highly specific for retina and represents more than 50% of the fatty acids in the rod outer segment (ROS). It appears that DHA is important in maintaining the normal structure and function of the  
30 retina (Anderson et al., 1992, Neurobiology of Essential Fatty Acids, Bazan et al., eds., Plenum Press, New York, pages 285-294). Increased dietary consumption of DHA and its precursor, eicosapentaenoic acid, from seal meat and fish has been

linked to an increased incidence of macular degeneration in Greenland Eskimos (Rosenberg, 1987, *Arct. Med. Res.* 46:64-70).

Certain delta 6 desaturases have been cloned from plants. For example, a delta 6 desaturase has been cloned from borage (Sayanova et al., 1997, *Proc. Natl. Acad. Sci. USA* 94:4211-4216). This delta 6 desaturase is unusual in that its cytochrome b5 electron donor is present as an N-terminal extension of the enzyme rather than being synthesized as a separate protein. The borage delta 6 desaturase has been shown to be functional, in that transfer of the cloned gene encoding it to tobacco results in the synthesis of high levels of GLA and octadecatetraenoic acid (OTA) in the transgenic tobacco leaves. GLA and OTA are the products of delta 6 desaturase activity on linoleic acid and ALA, respectively.

Based on its hydropathy profile, the borage delta 6 desaturase appears to be a membrane-bound protein. Examination of the amino acid sequence of the borage enzyme, as well as the amino acid sequences of membrane-bound desaturases from a wide variety of organisms, has revealed three regions of conserved short motifs containing histidine residues (HX(3 or 4)H, HX(2 or 3)HH, and HX(2 or 3)HH) having a conserved spacing from each other (Shanklin et al., *Biochemistry*, 1994, 33:12787-12794).

A DNA sequence has been isolated from sunflower embryos that, judging from its sequence, appears to encode a delta 6 desaturase having a cytochrome b5-like moiety fused to its N-terminus (Sperling et al., 1995, *Eur. J. Biochem.* 232:798-805).

#### SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences that encode a delta 6 fatty acid desaturase, cytochrome b5-related protein (CYB5RP). The present invention includes genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. The genomic CYB5RP DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The cDNA encoding CYB5RP protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2. Also provided is CYB5RP protein encoded by the novel DNA sequences. The CYB5RP protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3.

Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are methods of producing delta 6 unsaturated fatty acids using DNA encoding CYB5RP or using CYB5RP protein.

## 5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the enzymatic conversions involved in the linoleic acid (n-3) and alpha-linolenic acid (n-6) pathways of essential fatty acid synthesis.

Figure 2A-G shows the genomic DNA sequence of the CYB5RP gene (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon at position 544 in exon 1 and the stop TGA codon at position 18,103 in exon 12 are shown in bold. The putative polyadenylation signal ATTAAA located approximately 20 base pairs upstream of the polyA tail is shown in bold italics (position 18,373 in exon 12). DNA sequence upstream of exon 1 represents a putative promoter region of the CYB5RP gene., as indicated by the presence of the TATA box at position 353 (underlined bold)..

Figure 3A-C shows the cDNA sequence (SEQ.ID.NO.:2) and the amino acid sequence (SEQ.ID.NO.:3) of CYB5RP. The region encompassing amino acids 1-102 represents the cytochrome b5 domain. The region encompassing amino acids 182-186 represents HIS BOX 1. The region encompassing amino acids 219-223 represents HIS BOX 2. The region encompassing amino acids 383-387 represents HIS BOX 3.

Figure 4 shows a portion of the cDNA sequence (SEQ.ID.NO.:4) and a portion of the amino acid sequence (SEQ.ID.NO.:5) of mouse CYB5RP.

Figure 5A shows a Kyte-Doolittle hydropathy plot of CYB5RP. Figure 5B shows the proposed membrane topology of CYB5RP based on its hydropathy plot. This membrane topology is similar to that proposed for other membrane-bound fatty acid desaturases (Shanklin et al., Biochemistry, 1994, 33:12787-12794). The amino acids shown in Figure 5B are portions of (SEQ.ID.NO.:3).

Figure 6 shows the output of the Profilescan program from the Wisconsin GCG package. The upper amino acid sequence is from CYB5RP (positions 31-78 of SEQ. ID. NO.3). The lower amino acid sequence is positions 1-48 of the cytochrome b5 profile (SEQ. ID. NO.:6.). The output shows that CYB5RP

contains a profile typical for the heme-binding domain of the cytochrome b5 protein family. Importantly, the region of identity includes the invariant HPGG motif, where histidine represents a heme axial ligand for iron.

Figure 7A and B show the results of BlastP searches of the GenBank database using the full-length CYB5RP amino acid sequence as the query. Figure 7A shows the hit with highest homology, a hypothetical protein from sunflower. The sunflower protein and CYB5RP share three His boxes (boxed) in which the spacing between the His boxes is conserved. Also boxed is the HPGG motif typical for the heme-binding domain of the cytochrome b5 protein family. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.3. The lower amino acid sequences shown are portions of the amino acid sequence of the hypothetical protein from sunflower (Sperling et al., 1995, Eur. J. Biochem. 232:798-805). The sequence shown as positions 348-432 is SEQ. ID. NO.:7. The sequence shown as positions 22-74 is SEQ. ID. NO.:8. The sequence shown as positions 152-227 is SEQ. ID. NO.:9. Figure 7B shows the hit with the second highest homology, a delta 6 desaturase from *Borago officinalis* (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216). The *Borago* protein and CYB5RP also share three His boxes with conserved spacing, as well as the HPGG motif. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.3. The lower amino acid sequences shown are portions of the amino acid sequence of the *Borago* delta 6 desaturase. The sequence shown as positions 338-424 is SEQ. ID. NO.:10. The sequence shown as positions 12-64 is SEQ. ID. NO.:11. The sequence shown as positions 153-220 is SEQ. ID. NO.:12.

Figure 8 shows additional results of BlastP searches of the GenBank database using the CYB5RP protein as the query. Figure 8 shows the amino acid alignment between the CYB5RP protein and a delta 6 desaturase from *Synechocystis* sp. (strain pcc 6803) performed by the BlastP program. The *Synechocystis* delta 6 desaturase and CYB5RP share three His boxes, two of which are shown in Figure 8 (boxed). In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The CYB5RP

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sequence shown is a portion of SEQ. ID. NO.3. The *Synechocystis* sequence shown is SEQ. ID. NO:13.

Figure 9A shows the expression pattern of the CYB5RP gene in 9 human tissues, as determined by RT-PCR amplification with 21 cycles. Expression is  
5 detected in human retina, kidney, pancreas, placenta, and brain. Figure 9B shows the results of the analogous experiments performed with 25 cycles of amplification. Expression of the CYB5RP gene is seen in all the human tissues studied.

#### DETAILED DESCRIPTION OF THE INVENTION

10 For the purposes of this invention:

“Substantially free from other proteins” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a CYB5RP protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than  
15 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-CYB5RP proteins. Whether a given CYB5RP protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*,  
20 silver staining or immunoblotting.

“Substantially free from other nucleic acids” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, a CYB5RP DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%,  
25 preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-CYB5RP nucleic acids. Whether a given CYB5RP DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*,  
30 ethidium bromide staining, or by sequencing.

“Substantially the same biological activity as CYB5RP” means being able to introduce a double bond into the 6 position of linoleic acid under conditions in which CYB5RP is able to introduce a double bond into the 6 position of linoleic acid.

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TOP SECRET



A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

The present invention relates to the identification and cloning of cytochrome b5-related protein (CYB5RP), a gene which encodes a human delta 6 fatty acid desaturase. The gene is present on PAC clones 759J12, 756B3, 519O13, and 466A11 from an area of human chromosome 11q12 that has been shown to contain a gene related to Best's macular dystrophy (Cooper *et al.*, 1997, Genomics 41:185-192; Stöhr *et al.*, 1997, Genome Res. 8:48-56; Graff *et al.*, 1997, Hum. Genet. 101: 263-279). This linkage between the chromosomal location of the CYB5RP gene and the location of the gene related to Best's macular dystrophy can be used diagnostically by identifying restriction fragment length polymorphisms (RFLPs) in the vicinity of the CYB5RP gene, *e.g.*, in SEQ.ID.NO.:1. Such RFLPs will be associated with the Best's macular dystrophy gene and thus can be used to identify individuals carrying disease-causing forms of the Best's macular dystrophy gene.

CYB5RP was identified as an EST hit in sequence scanning data from PAC clones from human chromosome 11q12. In addition, a full length cDNA of CYB5RP was recovered from a human retina cDNA library. The genomic region of CYB5RP has been sequenced and the exon/intron organization of CYB5RP has been determined. The CYB5RP gene has 12 exons. The promoter region of CYB5RP was identified upstream of the 5' UTR by detecting consensus elements required for eukaryotic transcription. The expression pattern of CYB5RP was determined by RT-PCR analysis in 9 human tissues. The CYB5RP gene is expressed predominantly in human retina, kidney, pancreas, and placenta; lower levels of expression are also detected in brain, heart, lung, liver, and skeletal muscle. Bioinformatic analysis revealed significant homology to a group of plant and bacterial fatty acid desaturases. All of the typical amino acid motifs present in these fatty acid desaturases are also present in CYB5RP. Kyte-Doolittle algorithm analysis predicts a transmembrane organization typical of fatty acid desaturases for CYB5RP (see Figure 5). CYB5RP is

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unusual in that it contains a cytochrome b5 region in its N terminus. While many fatty acid desaturases utilize cytochrome b5 as an electron donor, most have not incorporated this cytochrome as part of their polypeptide chain.

That CYB5RP is a fatty acid desaturase is shown by the following evidence:

- (1) CYB5RP possesses significant homology to a group of plant and microbial fatty acid desaturases;
- (2) Like other fatty acid desaturases, CYB5RP has three conserved histidine boxes, with correct spacing between the boxes; and
- (3) The predicted membrane topology of CYB5RP is similar to that of known fatty acid desaturases.

That CYB5RP is a delta 6 fatty acid desaturase is shown by the following evidence:

- (1) CYB5RP contains a cytochrome b5-like moiety fused to its N-terminus. The only two fatty acid desaturases that contain cytochrome b5-like moiety fused to their N-termini are known or suspected to be delta 6 desaturases.
- (2) The only two plant desaturases that are known or suspected to introduce a double bond in the 6 position have an atypical His box 3 (QI/LEHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.
- (3) The only bacterial desaturase that is known to introduce a double bond in the 6 position has an atypical His box 3 (QVTHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.

CYB5RP is a target for the development of drugs for the treatment of disorders of lipid metabolism and for the treatment of conditions that require the modulation of the biosynthesis of prostaglandins and leukotrienes (asthma, pain, etc.). CYB5RP is also a target for the development of drugs for use in treating skin diseases, diabetic complications, reproductive disorders, including breast pain and premenstrual syndrome, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infections, and various forms of retinal degeneration, including age-related macular degeneration.

CYB5RP is homologous to a delta 6 desaturase from *Borago officinalis* (see Figure 7B). Both CYB5RP and this *Borago* delta 6 desaturase, unlike desaturases from higher plants, are unusual in containing a cytochrome b5-like

domain fused to their N-termini (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216; hereinafter "Sayanova"). The *Borago* desaturase has been expressed in transgenic tobacco, resulting in high levels of delta 6 desaturated fatty acids in the transgenic tobacco leaves, including high levels of  $\gamma$ -linolenic acid (GLA) (Sayanova).

- 5 Given the medical importance of GLA, Sayanova proposed that transgenic plants, expressing the *Borago* delta 6 desaturase, would be valuable as sources of GLA. Similarly, CYB5RP, expressed in transgenic plants, is expected to provide a valuable source of GLA.

The present invention provides DNA encoding CYB5RP that is  
10 substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding CYB5RP. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 2 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 12 exons. These exons collectively  
15 have an open reading frame that encodes a protein of 445 amino acids. When an alternatively spliced exon 8 is used, a CYB5RP protein of 433 amino acids, lacking amino acids 317-328, is produced. Thus, the present invention includes two cDNA molecules, encoding two forms of CYB5RP protein, that are substantially free from other nucleic acids. The first cDNA is shown in Figure 3 and has the nucleotide  
20 sequence SEQ.ID.NO.:2. The second cDNA is identical to the first, except that it does not contain the nucleotides at positions 1,019-1,054.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding region of SEQ.ID.NO.:2. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids  
25 having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2. The present invention also includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2, except that the nucleotides at positions 1,019-1,054 are missing. Also included in the present invention are recombinant DNA molecules having a nucleotide sequence comprising  
30 positions 71-1,405 of SEQ.ID.NO.:2 and recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 with the exception that positions 1,019-1,054 are missing.

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5 The novel DNA sequences of the present invention encoding CYB5RP, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which CYB5RP is not naturally linked, to form "recombinant DNA molecules" encoding CYB5RP. Such other sequences can include DNA sequences  
10 that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NO.:1 or 2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows:  
15 Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 hr in a  
20 solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at  
25 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor  
30 Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the

construction of synthetic DNA that encodes the CYB5RP protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:2, but still encodes the same CYB5RP protein shown as SEQ.ID.NO.:3. Such synthetic DNAs are intended to be within the scope of the present invention. Also with the scope of the present invention are synthetic DNAs that encode a CYB5RP protein lacking amino acids 317-328 of SEQ.ID.NO.:3.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding CYB5RP protein. Such recombinant host cells can be cultured under suitable conditions to produce CYB5RP protein. An expression vector containing DNA encoding CYB5RP protein can be used for expression of CYB5RP protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, plant cells such as tobacco, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of CYB5RP protein and which are commercially available, include but are not limited to, L cells L-M(TK<sup>-</sup>) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

A variety of mammalian expression vectors can be used to express recombinant CYB5RP in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNA1 and pcDNA1amp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, CYB5RP can be purified by conventional techniques to a level that is substantially free from other proteins. A description of vectors that can be used to express CYB5RP can be found in, e.g., Goeddel, ed., 1990, Meth. Enzymol. vol. 185 or Perbal, 1988, A Practical Guide to Molecular Cloning, John Wiley and Sons, Inc.

The present invention includes CYB5RP protein substantially free from other proteins. The amino acid sequence of the full-length CYB5RP protein is shown in Figure 3 as SEQ.ID.NO.:3. Thus, the present invention includes CYB5RP protein substantially free from other proteins having the amino acid sequence

5 SEQ.ID.NO.:3. Also included in the present invention is a CYB5RP protein that is produced from an alternatively spliced CYB5RP mRNA where the protein has the amino acid sequence of SEQ.ID.NO.:3 with the exception that amino acids 317-328 are missing.

As with many proteins, it is possible to modify many of the amino

10 acids of CYB5RP and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified CYB5RP proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as CYB5RP. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein

15 (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, *Science* 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as

20 CYB5RP. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments

25 where the above-described substitutions do not occur in the His boxes of CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the sunflower protein depicted in Figure 1 of Sperling *et al.*, 1995, *Eur. J. Biochem.*

30 232:798-805 when these two proteins are aligned by BLASTP analysis. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the

CCCTCTACCCCTGTCCCATCAGGC (SEQ.ID.NO.:15)

One of skill in the art would recognize that many other primer pairs based upon SEQ.ID.NO.:2 would also be suitable.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl<sub>2</sub>, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA template, 0.05 units/μl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press .

A suitable cDNA library from which a clone encoding CYB5RP can be isolated would be Human Retina 5'-stretch cDNA library in lambda gt10 or lambda gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of either 445 amino acids (SEQ.ID.NO.:3) or an open reading frame of 433 amino acids (SEQ.ID.NO.:3 lacking the amino acids at positions 317-328) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). CYB5RP protein can then be produced by transferring an expression vector encoding CYB5RP or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. CYB5RP protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding CYB5RP can be isolated from a cDNA library using as a probe oligonucleotides specific for CYB5RP and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described

in, *e.g.*, Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for CYB5RP and that can be used to screen cDNA  
5 libraries can be readily designed based upon the cDNA sequence of CYB5RP shown in SEQ.ID.NO.:2 and can be synthesized by methods well-known in the art.

Genomic clones containing the CYB5RP gene can be obtained from commercially available human PAC or BAC libraries available from Research Genetics, Huntsville, AL. PAC clones containing the CYB5RP gene (*e.g.*, PAC  
10 clones 759J12, 756B3, 519O13, and 466A11) are commercially available from Research Genetics, Huntsville, AL (Catalog number for individual PAC clones is RPCI.C). Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the CYB5RP can be isolated, using probes based upon the CYB5RP sequences disclosed herein. Methods  
15 of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).

The present invention also provides oligonucleotide probes, based upon SEQ.ID.NO.:2 that can be used to determine the level of CYB5RP RNA in a sample. In particular, the present invention includes DNA oligonucleotides  
20 comprising at least 18 contiguous nucleotides of SEQ.ID.NO.:2. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide probes can be packaged in kits.

In addition to the utilities described above, the present invention makes possible the recombinant expression of the CYB5RP protein in various cell types. In particular, it is advantageous to recombinantly express CYB5RP in plant cells. Such  
25 expression in plant cells provides a method for the production of high levels of valuable EFAs such as GLA and OTA in the recombinant plant cells. An example of such recombinant expression of a delta 6 fatty acid desaturase, in that case from borage, is described in Sayanova *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94:4211-  
30 4216 (Sayanova). The recombinant expression of the borage delta 6 desaturase led to the production of high levels of GLA and OTA in the leaves of the tobacco plants in which it was expressed. The procedures described in Sayanova can be easily adapted to express CYB5RP in tobacco, thus providing an additional, useful way to produce



The present invention also makes possible the development of assays  
5 which measure the biological activity of the CYB5RP protein. Such assays using  
recombinantly expressed CYB5RP protein are especially of interest.

15 Such assays may comprise:

- (a) recombinantly expressing CYB5RP protein in a host cell;
- (b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;

In particular embodiments, the biological activity of the recombinantly expressed CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid or alpha-linoleic acid.

The present invention includes activators and inhibitors identified by the methods described herein as well as pharmaceutical compositions comprising

such activators and inhibitors. The activators and inhibitors are generally combined with pharmaceutically acceptable carriers before use to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the activator or inhibitor.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where CYB5RP activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route

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Gene therapy may be used to introduce CYB5RP polypeptides into the cells of target organs, *e.g.*, the pigmented epithelium of the retina or other parts of the

- retina. Nucleotides encoding CYB5RP polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding
- 5 CYB5RP polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with CYB5RP polypeptides will be particularly useful for the treatment of
- 10 diseases where it is beneficial to elevate CYB5RP activity.

- The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the
- 15 scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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## WHAT IS CLAIMED:

1. A recombinant DNA molecule encoding a polypeptide having the amino acid sequence of SEQ.ID.NO.:3.
2. A recombinant DNA molecule comprising a nucleotide sequence selected from the group consisting of:  
SEQ.ID.NO.:1;  
SEQ.ID.NO.:2;  
SEQ.ID.NO.:2 lacking positions 1,019-1,054;  
positions 71-1,405 of SEQ.ID.NO.:2; and  
positions 71-1,405 of SEQ.ID.NO.:2 lacking positions 1,019-1,054.
3. A DNA molecule that hybridizes under stringent conditions to the DNA molecule of claim 2.
4. An expression vector comprising the DNA of claim 1.
5. A recombinant host cell comprising the DNA of claim 1.
6. A CYB5RP protein, substantially free from other proteins, having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:3 and SEQ.ID.NO.:3 lacking positions 317-328.
7. The CYB5RP protein of claim 6 containing a single amino acid substitution.
8. The CYB5RP protein of claim 7 where the substitution is a conservative substitution.
9. The CYB5RP protein of claim 6 containing amino acid substitutions where the substitutions do not occur in positions where the amino acid

present in CYB5RP at those positions is also present in the corresponding position in the delta 6 desaturase from sunflower when CYB5RP and the delta 6 desaturase from sunflower are aligned by BLASTP analysis or where the substitutions do not occur in positions where the amino acid present in CYB5RP at those positions is also present  
5 in the corresponding position in the delta 6 desaturase from *Synechocystis* when CYB5RP and the delta 6 desaturase from *Synechocystis* are aligned by BLASTP analysis or where the substitutions do not occur in positions where the amino acid present in CYB5RP at those positions is also present in the corresponding position in the delta 6 desaturase from borage when CYB5RP and the delta 6 desaturase from  
10 borage are aligned by BLASTP analysis.

10. An antibody that binds specifically to the CYB5RP protein of claim 6.

15 11. A DNA or RNA oligonucleotide probe comprising at least 18 contiguous nucleotides of at least one of the sequences of claim 2.

12. A method for determining whether a substance is an activator or an inhibitor of CYB5RP protein comprising:

20 (a) recombinantly expressing the CYB5RP protein of claim 6 in a host cell;

(b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;

25 where a change in the biological activity of the recombinantly expressed CYB5RP protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CYB5RP protein.

30 13. The method of claim 12 where the biological activity of CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid.

14. A pharmaceutical composition comprising an activator or an inhibitor of CYB5RP.

- 5 15. A method of treating macular degeneration comprising administering to a patient an effective amount of the pharmaceutical composition of claim 14.

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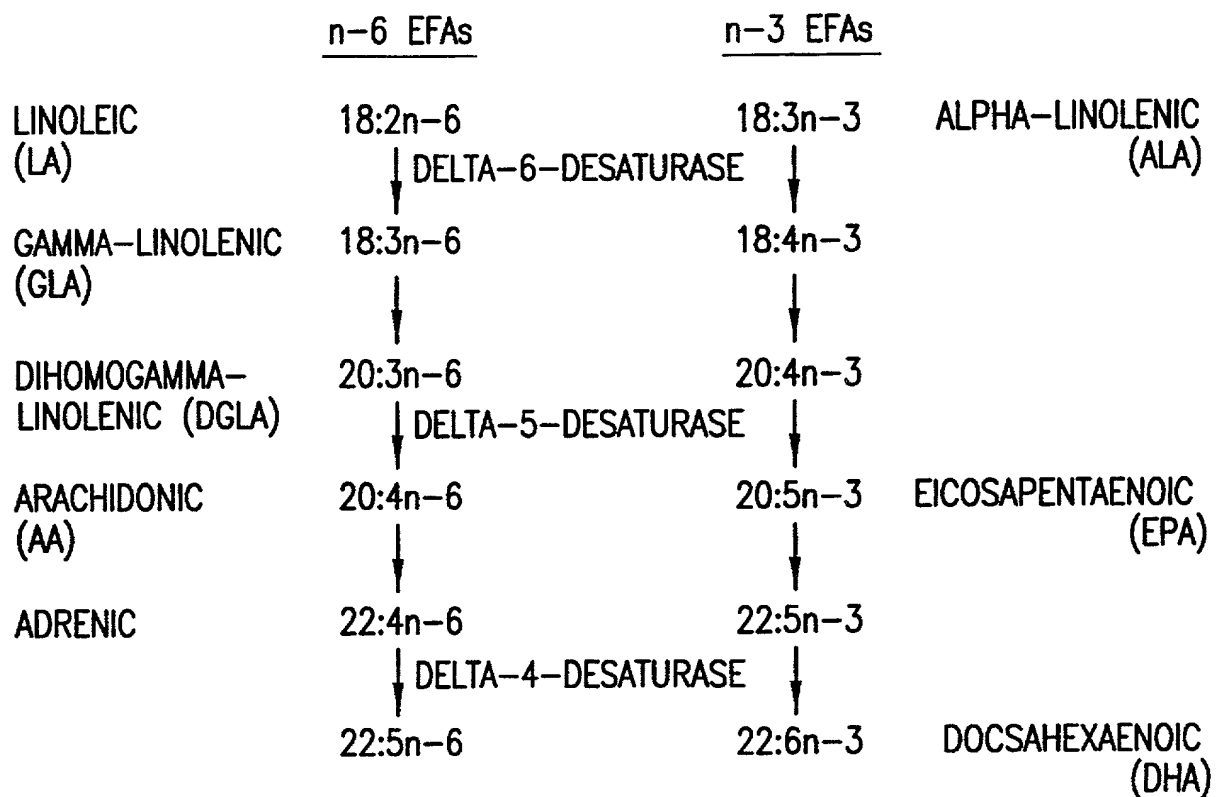


FIG.1



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1 gctcacagac cgggactccg cctccggttc ccgagggcgt ggcgagggcg  
 51 tgcgggacgc ccaacaggtg cgtgttgtgt ccccaggccc cgcgctccgg  
 101 gtggagtcaa gagcctggaa gccggcagcc cgggaaaagg gggcgggacg  
 151 gtgccccggg gcagggctgg gtggcgcccg ctgtcctccc gggaggggag  
 201 ggccgcctcg acgccgcctt ccttggcgcc caatggagac cgaggccccg  
 251 cgcctggatt ggagcggacg cgggggtcag ccagccttgg gggccggggc  
 301 ctggccgggg gcgggggggc aggcgaggcg aggcggggcg cgtccgcgcg  
 351 gttataaaggc ggggagttcc ctgcgccgag agccgggagg cgcacgctcg  
 401 ctcgtacggc ggccgcggcg gcagggcggg gccggagcag cgggcggcgg  
 451 cggaggcggc gcccgggagc gctCTTCGCT TCCCTCGGGG TCTTGCTCGG  
 501 ACCTCGGCCA CCGCCTGGGA TCCCCAGGAC TCGTGCGTGC AGCATGGGCG  
 551 GCGTCGGGGA GCCGGGACCG CGGGAGGGAC CCGCGCAGCC GGGGGCGCCG  
 601 CTGCCCACCT TCTGCTGGGA GCAGATCCGC GCGCACGACC AGCCCCGGCGA  
 651 CAAGTGGCTG GTCATCGAGC GCCGCGTCTA CGACATCAGC CGCTGGGCAC  
 701 AGCGGCACCC AGGGGGCAGC CGCCTCATCG GCCACCACGG CGCTGAGGAC  
 751 GCCACGgtaa ggaagccata aggaagccac ccaccggcgg gtggagcctg  
 801 gagctcggtc gtgggcgtga tgtcccgctc cacctgtggg gccttagcat  
 851 cctccctccc ctgcgtgacc tttgacctcc acgccgggac ccagagttgg  
 901 ggtggactag ccagggccag atgtggggta gggagggcag ttccttgcgt  
 951 ggaggaccgg cagctgtcca cggagcaggt ctgcggggga ggagggggcc  
 1001 tcagaggtgg gtgtgtcatg ctgcagagcc tgccctgggt gaggggctgc  
 1051 cctgttgctc ccaggtccct gtttcagttc tgggtcccca tgctgggtgc  
 1101 ttgctgagtg ctaggggtag ggcagggcag ggtccccagg ggccggtaag  
 1151 gacatgccat tagaggctgg gggctgggcc ggcctgaggt ctgtggcttt  
 1201 cccaagagct tctgtaaagg gctcaggagc agtgactcac ctctccgggc  
 1251 tagcagctgc acgtgggagg gctttgccag ccaggctggg tggccctctc  
 1301 ctggaagcac agtcacccca ggaacaggct ggccctggg gaccccaact  
 1351 tcccaatccc agccctgtc tagacaggca gggatgtagc ctggccccag  
 1401 ggtactgtct ggctggagtc cagtgggtga gcagcccgac cagccctttt  
 1451 tccttagtta cccacctgca taataggggt tggggccacg atgccctgtc  
 1501 cttgaccctc caaatttcta ggttggccac actgggtatc aggaaggtct  
 1551 tcaagaccgg aggacatgaa tctgaatgc tggctttttg ggcagcagcg  
 1601 gaggttctgt ccagtccag gactgtcggc gtccctcttg ccagggccac  
 1651 ctgctctctg ccgattgcca tctccagcat gttggacaat ctctactgga  
 1701 ctctttgagg aagaaagccc ctcttttccc tttccacccc atgaagctga  
 1751 ggagtggaga taagaatcct cctgaaattc taaaaaaga aaaaaaaaaa  
 1801 aaagagaacg ccttgtccgt ggctgttcag gcgccagacg ctggcccgag  
 1851 gggacagcac agccgtggga tgaagcagcc tgggggcagt atttgagcgt  
 1901 gcaggtgttt gcatgtctgg gtgagtggtg tgtgtgtgcc tgcccttctg  
 1951 ccagggcggt gcgaggtgag gggcacggct tctcccaaa ggccttgctg  
 2001 agccctggcc tcccttcaag gactcttggt gatgcctgct ctggtctttt  
 2051 tttaaaaaag tatctatttt atttattatt atttgtttaa aaatagagac  
 2101 aggggtctcac tatgttgctc gggctggtct caaagtcctg ggttcaagca  
 2151 ttcctcctgc ctccagcctc gaaagtctct ggattacagg catgagccac  
 2201 cactcccggc ctgctctagt cttttgtaac cttagaggaca gtatggatac  
 2251 agaaaacttt actcccacc aaccgccgga gacagagtct tgctctgcca  
 2301 cccagactgg agtgcaatgg cgccatcttg gctcactgca acctccgcct  
 2351 cccaggttca agcgattctc ctgcctcagc ctcccgagta gctgggatta  
 2401 cgggcacgcg ccaccacgcc cagcatattg tatttttagt agagacgggg  
 2451 tttcaccatg ttggccaagc tgggtctcgaa ctccctgacct cgtgatccac  
 2501 ccacctcggc ctcccaaagt gctgggatta caggcgtgag ccaccacgcc  
 2551 cggctgggat acagaaagct tttatttcat cactgtttcc tgcctggtgc

FIG.2A

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2601	caggcccatg	ctgggggttcc	tcccaagtgg	aattactgac	ttaacatttta
2651	gcttgggatc	ctgagacttc	catcacacag	ttttctcatt	gattcgcagc
2701	caataatata	tgttttaaaa	acatctcagg	cagagcgctg	tggctcacac
2751	ctgtaatccc	agcacttttg	gaggctgagg	tgggcagatc	acctgaggtc
2801	gggagtttga	gaccagcctg	accaacatgg	agaaaccctg	tctcttctaa
2851	aaaaatacaa	aattagccag	gcgtggtggc	gcatgcctgt	aatcccagca
2901	ctttgggagg	ctgaggcagg	agaatcgctt	gaaccagga	gacggagggt
2951	ccggtgagcc	gagatcgcg	cattgcactc	cagcctgggc	aacaagagca
3001	aaactccgtc	tcaaacaac	aaacaaaaa	catctctctg	ctccttgggg
3051	ccgggtgcca	gctctgctat	tggaggcact	gagcgacctt	gaagcaggca
3101	tgtcactcct	ctgtgcccc	gtttactcat	ctgtaaagtg	ggagagctgg
3151	ggcagacagt	gagctggctg	agggcaggac	tgtgtctcct	caagcccatg
3201	gccaggggct	gccaggtagt	agtttgtatt	cggtaaattg	tgtgtggccc
3251	taagtgtgag	cgtgccctgc	aaactgcagc	gtatggtggg	acagccctgc
3301	acggctaccc	ctttcctggg	tgaccttatt	tggttacggt	cctatctgaa
3351	gtaggaaagg	gacactttag	gctgtctctt	agctccctca	aggccccaca
3401	gcctggacta	gagttgccag	aaataacttg	tccattcagg	ccaaagggac
3451	tgtgaggttg	ctgggatggg	gcaatcagtc	tttgtccatg	atgaaccac
3501	agggtagacc	aggggttggg	ccagcccatg	gccctgtgta	gttgagccca
3551	ggccccaggc	atcccatccc	gggcggtggc	ctcaggtgga	ggtggggcag
3601	ccagttgcca	gggatgtgtt	ccagcgggtc	cctctacca	gccccggctg
3651	cccatcagct	gttctcaagt	ccaggcaatg	aagccttctt	gccaggaaat
3701	tcccagagtt	tctgtgccat	gaagtcagcc	tgtggccatc	ttgggacaca
3751	aggccgggtg	ccctggggag	agtactctgg	gcccttggcc	aggtttgtct
3801	gagagtcata	ggcagcctga	tactagtga	gccagccagg	gagggatgag
3851	gccagccgc	tgctggccat	aagtatataa	gggccatgtg	gtgagtcctt
3901	actatgtgcc	aggttttgaa	atcagtaact	gatttattga	aaccctctct
3951	tttaatcctc	aaggtgcccc	tatgaggcac	gtaccattta	ttgttattgc
4001	cacttgacag	atgagaaaac	agaggctcag	agaggcaaag	tggcttgaaa
4051	ttcagtgatt	ggtctgggat	ttgaatccac	agccatgttc	tttaaggcat
4101	gctatgctgc	cacctatcct	gtttatttcc	ggcactcatt	gattcttcaa
4151	tgtttgactc	attaaatcca	tcagttagca	tcttctctgt	gtcatgcatg
4201	gttctcacct	ctgaagatgt	agctgtgagc	aaaacttcta	caggggaatga
4251	gttcacagca	gagggatcag	ctagagcaaa	ggctcagagg	tgggaccgtg
4301	cgtcctgtgt	tccaggaata	cagtatggct	gcagcagaga	gcagtggaga
4351	gagggcctgg	cagtgaggtc	tagaggcggc	cgggctggct	catgctggat
4401	gtttgtgtcc	tcggaaggac	tttggcttta	ttttaagag	gatggggagc
4451	cccagagagc	acagcaggga	agcctgggga	gtctgatgga	catttaaaag
4501	gatacctaat	ggagagagtg	aaggcagagc	cttccagaag	ggtaagagaa
4551	gggaggatgg	agacctgccc	tcccccaagg	gaggccactc	agaagaggta
4601	gagtgtggcc	agggcagaga	gcaagagagg	ctgtggacac	aggcacactg
4651	gtccagttag	agccattaga	cacattagat	ttagcttcat	gttgtcttta
4701	gagagggagc	cagcctggcc	tcgctctatg	atcttggaca	catcctttca
4751	cttctgggtc	tcagtttccc	cattagtgtg	atgaggatga	gaatgctttt
4801	gtcctgggca	cactatgagg	gtggtgctgg	gcacctgggt	gcctgggttac
4851	catgggcaac	aaagctctat	tcattgggtg	ggtgaatgca	ttgccacag
4901	caactcaggg	cggatgagga	gtttcccagc	agccctgggt	gccctttcgg
4951	ctgaagccct	aacaactgtg	ggaaaatcca	agttccagca	gacccctga
5001	gccctctgcc	ttaggacct	ccttctaggt	ggttctctga	gcctggcctg
5051	agctggagga	gggagtggcc	agtgtgtcag	cagaggctgc	ttcatagtaa
5101	ttgcagccaa	cagttattga	ctaggcactg	ttctgagggg	tttagatgtg
5151	gtaactgatt	gaattcgctt	aacaacttta	tgaggttaagt	cctattgtta
5201	gcccattttg	tagatgagga	gactgagttt	gaaactgggg	ggtgtaatgg
5251	aaccttctca	ggacccttga	agggtagggc	ctttgtactc	gggccacgag

FIG.2B

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5301 ggtgggggttt gtgtctgggt gggagctggg gagggacagg actaggatta  
 5351 ggcagatctg aggccacagg agttggttgg ggggtggctc cagagccact  
 5401 ccaactccctc ctaccacatt gactgccttg aaagtccctc aatggccact  
 5451 cccatgaagt gtgactgctc tgggctcccc gcaggcgttt tctgcaaggc  
 5501 caccgcccac ccaggccctc tccccagagg ggctgcagtg ccttgctcct  
 5551 tccttggtggg aagagttggg attgtctggc gtcagcagga tactgccctc  
 5601 gggcatccct cccggtctct tcctgcgggt ttctgatgaa acagccaggc  
 5651 tccagtagtg gagccagagg tcagtgggtg agagaggacc aggagccaga  
 5701 gggatatagct gctttggggc tactgtgggg tcagggacac ttgtgaggcc  
 5751 aagcgtcctg gctgcaggag cctcacata tatgccacc cttcaccagg  
 5801 acattgaggg gtgctggggg acaggggtag ctttttgggg gtgtctgcct  
 5851 tcgacttggg ctccgctaca caggccaaat ttggatgtcc catgtttaga  
 5901 gctgtgtttc tttgggacct cttggggcct cagtttcctc atctgtaaaa  
 5951 tgggatactg atagtgttc cccactggcc tcctctgacg ggcgccaggg  
 6001 agaggatggg acggagcatg gtgtgctggg cacgctcctg ctgtaccac  
 6051 ccacctggga gaggggagag gcaggaatgt cctgggggtg tcctttgagg  
 6101 catagccctg tcacccaac atcctacaaa ggcatgagaa ggcagcgagg  
 6151 acagaccccc accacctgag cctcagcag cctgccaca ctccctgctt  
 6201 caccaccttc ctgactgatc tggcacattc ttgattctcc tagggagtga  
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 6301 cagccctcc tctctccc cctcaggctt ggccaggact taacaggcag  
 6351 gcagagaagc agcttctcca ctctcttccc tgacacctgt aggccctcc  
 6401 tgcaggcact tacctctaag tggactctca ggaggaggct catcagggtc  
 6451 gcagggtcga gaaagagctg ggctgtggag ctcttgccaa ccgccaggcc  
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 6551 agatggggat ttgtggttcc cagtttactg atgagaaata ctgatgagag  
 6601 atgggtgtgg tcttgtctgg ggctccctgg ctccctggata gcagctcagg  
 6651 ttccatcctg ggcaggctgg ctctgggaca ccccccgac cagctgctgt  
 6701 gtgggattca cggtggggct tgggcagggg gtgggatctt ggggccaaact  
 6751 gagccactct aggcttccag ggaccaaggc caggctgagc tgtctctgta  
 6801 tcctgagaga gcatgaacat cacagaagat gggcccggtt tcgaatcca  
 6851 gctctgccac tactaactgg gacctgggca ggggtccctt cccgctgagc  
 6901 cttcatttcc tcaccagcaa aatgggttcgt gccctgctt tgggggctgt  
 6951 ggagggttgg ctcttgtcta cttgttcata cctgctgttg agcagctgct  
 7001 ctgtgcccggc ctctgaggat gccactgtga acagagcctg tcgctacctc  
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 7151 tgggtgctg tgggggttgg ggggacaggg ctttgtgcac gtagccctgg  
 7201 gtggatgttc ctgggtgcac ttagggtgtg tgagggtggg acctcccaca  
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 7301 agcccaggct cccagcagct gggcccttgg cttcttgaga tagtgactgg  
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 7401 tctgttccag gagtggcgac aagcacagtt tttcgctttt gtttttgttt  
 7451 tcttcacttt aagttccggg aaacgtgcag aatgtgcagg tttgttacat  
 7501 aggtatacat gtgccatggt ggtttgctgc acccgtaac cctcatcta  
 7551 ggttttaagc tccatataca ttaggcattt gtcctaagtc tctccctccc  
 7601 cttgcccctc acccgcccag taagccccgg tgtgtgatgt tcccttcctc  
 7651 gtgtccatgt gttctcattg ttcaactctc acttatgagt gagaagagac  
 7701 ctggactctg atctaacctc ggtcaaattg aactgtgtga ccttgaagaa  
 7751 gtagcttaac ctctctgagt cttagcttct gcctggcacc cccatcctta  
 7801 aggagaggcc cacagaggac cagggtcacat gacctcagcc agttccagag  
 7851 aaggctgttt gcttccaggt ttcggcctga gtccaggccc ctgccctact  
 7901 cgcactccct gatagcatga gaagcacagc cccagggtgc ccaccagct  
 7951 ctgagagccc agcctgcttc ccagggaact gtcacagccc cacctgtccc

FIG.2C

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8001 ttccccagct ggagccctgt caatggcttt ggggttctct gacacagccc  
8051 tgagggggct cacacttccc cttatcattg caaggggtag atctggcttg  
8101 aaggccctgg ggcaggcttg gttctgtcct cccctgtcag tgcctcgaca  
8151 gggctggcct ggggtgaatca ggaccaacgg gaaaggaggc gaggagacca  
8201 atctggaccc aagatcctca gctcaataag gtggccccag aactgacatg  
8251 gggatgata ggggaagggt gggaggagg agattctggg gccgcagcca  
8301 cagcttgac gttgcgccgg gtgtgtctgt gcgtgccagc tgcattcttg  
8351 cgtaccatgt gtgcaaggct gtgtttggct gagtgttcat gtgggccgtg  
8401 attgtgggca tgtttctgag tgtctgagt atgcctgctg gtgtgggctg  
8451 gtgggtgtgt ctgcatgtgc gtgtgtgtct ggggagtttc aaaggagaaa  
8501 gagggactca ccatcacgct ggctcagcct taaaaaggta ggacatcctg  
8551 acacgtgctg caacatggat ggaccttaag gacattgtgc tgagtgaaac  
8601 aagccagagg caaaggaaaca aacatgtgat ttctcccaga tgaggtttcc  
8651 ggaggaggca gatctgtatg gacagaagg agcatgggtg ttgccggggc  
8701 agggggagga gagaatggag aattagtgtt taatggggac agagtttcag  
8751 ttggggaagg tgaaaagggt ctggagctgg atgatgggtg tggttggaca  
8801 acactgtgca tgcacttaat accactgagc tggacaccta aaaatgctta  
8851 caatggtaaa tttcatgtat attttactac aatttttaaa aaattggctg  
8901 ggcgtgggtg cttatgcctg taatcccaac actttgggag gccaaaggcgg  
8951 gaggattgct tgagctcagg agttcaacac cagcctgggc aatatggtga  
9001 aaccccgact ctacgaaata taaaaaatt agcctggtgt ggtggcttgc  
9051 acctctaata ccacctactc agtaggctaa ggcacaagaa tctcttgaac  
9101 ctgggagggt gaggttgtag taagccgaga tcatgccact gcaaccagt  
9151 ctgggcgaca gagcaagact ctgtctcaaa aaataaaaaga taaataaaaa  
9201 aattagaggc caggtgtggc tcacacctgt actctcaaca ctttgggagg  
9251 ctgaggtggg aggatcgctt gaagtcaggc atttaagaca tgcctaggca  
9301 acatagttag accttgactc taaaaaaaaa ttcaaaagtt aatgagacat  
9351 ggtggcatgt gcctgtagtc ctgactgctg gggaggctga ggtgggagga  
9401 tcacttacga ccaggatttc aaggctgcag tgagctgtga ttgcatcact  
9451 gcactccagc ctggtgacag agtgaggccc tgtctcaaaa aaatttttca  
9501 gtgtttttct gggctgggag tgggtggtca ttctgtaat tccagcactt  
9551 tgggaggctg aggtgggtgg attgcttgag cccaggagtt taagaccagc  
9601 tgggcaacat ggcaaacctc atctctacaa aaaataaaaa taaaaaatta  
9651 gctgggcatg gtggtgcaca cctgtactaa cagctacgag agaggctaag  
9701 gtgggaggat cacctgagcc cgggagggtg aggctgcagt gagccatgat  
9751 tgcaccactg cactctagcc tgggcgatac agcaagacct tatctcaaaa  
9801 aaaaaaaaaa aaaaaaaaaa aaaaacaccc agtggggtca gtagaacccc  
9851 aagagtcttc ttccctccca gctccctgt acaccagccc cagctctgca  
9901 ggtagctggg ggcccagaca gcttcctggg gacccccagc cttccctctg  
9951 cccttttttc taccagtttt gctgcccctc cttcaagact catgtccaga  
10001 gggggtgaga tctgcactta tacagccccc tctctgttaa tgagttagcc  
10051 aagtcagccc aggttatttc agaaggggca cctaccagc cccccagtc  
10101 ccaagctgcc ctgggcctat aaaagcaggc aaggggaccc ctagtatgc  
10151 atgtagggtg tacctcttag tgggtgctgg aggggcctga agtgctttct  
10201 tccccagggt tggtaggaga atgtcctggc agtgacttca gggcccgctg  
10251 tcacttccgt ttttaagactc accagctggg aggctcatta gcaagaggac  
10301 aataggaggc ccctgtcctc agtcagcttt cttcaaagg gtttccttta  
10351 gcaactggga ggctccctt ctccagacct atggggacaa caccaccag  
10401 ctactgggtc tataagctgc tgtatggctc tggctagccc attcagagaa  
10451 agcctctgaa agtacaagga aaaaaatcag tccaagagct gtgaacaatt  
10501 agtgagccga ttacaatacc aagaccacag gcagacctgg aaggctaagt  
10551 gagccaggt gtgaagttca agcttacttt acttctgggc cacttcctgg  
10601 ctggtctctt tccctggccc ttatctttct cctggtctgt cttctcttct  
10651 caccctctt ctttactctt tcttccttct cctgcatcgt actccacccc

FIG.2D

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10701 cactccagct attacacaga atcgcgagaa tgttgaggatta ttcattttat  
 10751 ttatgatgtt ttcttttttg taaaaataga gacaaggtct cactatgtgg  
 10801 cccaggctgg tcttgaactc ctggcctcaa gcaatcctcg tgccttggcc  
 10851 tcttacagtg ctgggattac agatgtgagc caccatgcct ggccattttt  
 10901 atttacttta aaaaaaaaaat taggctgggc gcggtggctc acacctataa  
 10951 ttccagcact ttgggaggcc aagggtgggca gatcaactga ggtcaggagt  
 11001 taaagaccag cttggccacc tggggtcagg agtttgagac cagctactcc  
 11051 ggaggctgag accggagaat tgcttgaacc caggaggtag aggttgcaat  
 11101 gaactgagat catgccattg catgccagcc tgggcaacag agcaagactg  
 11151 tctcaaaaaa aaaaaaaatt atgtttttgt ctctgtcttc ctgctttgta  
 11201 agtcaaatca gtttaactgt tcaagtgtct tcttgcaaa ccccaagga  
 11251 ctcaatgtgt gtcgcccttg actgatcccc ccgccccgtg acccagtggg  
 11301 cctcagttcc aggttttccc acctaccctt caccactgc ttatgtttat  
 11351 aaaaacgggg taaatcaaat gttcgtgacc cagatcttat tctacatgca  
 11401 gtggaaactt gtatgactta agcttttttg aaaagcagaa ccttttttcg  
 11451 tggttcaaga aatcaagtc tccccgggag gtctttctgt aaatccagag  
 11501 ctgcagatgt ttgaccgtgt tcagagaggg gcccttgtgc tgggtgaagt  
 11551 ggatggggca cagcaggcaa tgggtgaaaa gcaggacaac ctggggccct  
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 11651 ttcctgtccg cctgtcgtct gctctgcca tccatccgta gtccttccgc  
 11701 ctgtctctgc tgggtgccc tgtgctactc agctgtgtct gtctgtccgc  
 11751 ctgactgtct gctctccttc agGATGCCTT CCGTGCCTTC CATCAAGATC  
 11801 TCAATTTTGT GCGCAAGTTC CTACAGCCCC TGTGATTGG AGAGCTGGCT  
 11851 CCGGAAGAAC CCAGCCAGGA TGGACCCCTG AATgtgagcc agagccctag  
 11901 gagaggctca gcccctgagg gagggggatg gctggagggc tgggagacat  
 11951 tgccacatgg ccaggagcag ctccctcggc attcgcccaa ggggatgcag  
 12001 agccagggtc gagcctgcc tcccctccca gggggcaggc agttgaaagt  
 12051 gaagctgtag ggatgccctg agaagtccag ggctccagat ctgggttagc  
 12101 caggcactcg tttggatccc gaggcaagct ccctccctgt tgcgcccag  
 12151 tgtcccatc aaaaggagga ttttgatgaa ctgatttctc tcttggtgt  
 12201 agcgtcttac ccaccccata ctttttggga gggagaggag gcttcaccac  
 12251 cagccagtgc tccagctcac accccgggct gggactctt gtcacttcat  
 12301 tctcttttgc ccacaccct tgggcctggc gatgggagga gcggtggggg  
 12351 ctccaggaga atgggggtgg ggaggaattt ctccctggc tgatcggccc  
 12401 ctctgctatg gcagGCGCAG CTGCTCGAGG ACTTCCGAGC CCTGCACCAG  
 12451 GCAGCCGAGG ACATGAAGCT GTTTGATGCC AGTCCCACCT TCTTTGCTTT  
 12501 CCTACTGGGC CACATCCTGG CCATGGAGGT GCTGGCCTGG CTCCTTATCT  
 12551 ACCTCCTGGG TCCTGGCTGG GTGCCAGTG CCCTGGCCGC CTCATCCTG  
 12601 GCCATCTCTC AGgtgacccc agttctgtgt tgcagccacc ttaactgccc  
 12651 aacagacgtg ggcccccatg catctgggca ttgtgaacat atttgctaaa  
 12701 tgaatgaatg gacctatgaa aggatgaatg gatgaataaa cagatgaatg  
 12751 agtgaacagt ctgaaggccc atcaggcatg tctgtgggtc aagctgcatt  
 12801 ccagatgagc caagaagttc cttcttgaac agattccgat caagcacagg  
 12851 gccactgagc cagaggctgc tgccctgcag cttcatgaca cttacgagcc  
 12901 cctccacctc cttgggactc agttctcatc tgtaaaaaga ggacactggc  
 12951 ccacaagggt cttgaaatgg agcattagca cgggggtacc ctgcaagctg  
 13001 aaaggattca ctggggcccc aggcctggc gggctccgtc ctcccaaca  
 13051 gcttctgacc ctgcctctct cccagGCTC AGTCTGGTG TCTGCAGCAT  
 13101 GACCTGGGCC ATGCCTCCAT CTTCAAGAAG TCCTGGTGGA ACCACGTGGC  
 13151 CCAGAAGTTC GTGATGGGGC AGCTAAAGgt gaggggtgggg tgggtggtca  
 13201 gccaggtgct gggtggcgct gggctctgcc aagtgtgtgg gcacagtcgg  
 13251 gggcacagcc tgccctgaga gcccctcct cctccacagG GCTTCTCCGC

FIG.2E

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13301 CCACTGGTGG AACTTCCGCC ACTTCCAGCA CCACGCCAAG CCCAACATCT  
 13351 TCCACAAAGA CCCAGACGTG ACGGTGGCGC CCGTCTTCCT CCTGGGGGAG  
 13401 TCATCCGTCG AGgtgggtgg ggagggacct ggacaacctc tggctgggcc  
 13451 tgcagctgag ggggagctaa tgcactgggt cccactctg cccctgacct  
 13501 agcccctgat ctggcctcca ctctggctgg gccaaactct gccccctgtg  
 13551 ctttccttcc cacctcccaa cctgctgggg acgaccagcc cgcttgctag  
 13601 aatctagagt tgcctttgac ccttggtccc agccagcccc gtgaccttgc  
 13651 ccgggagaag gaggtggcct ggagagctgc tgtctccagc cgccgcctgt  
 13701 ctccacagTA TGGCAAGAAG AAACGCAGAT ACCTACCCTA CAACCAGCAG  
 13751 CACCTGTACT TCTTCCTGAG tgagtgtcca tctgtccttc tgggggtggg  
 13801 gagtgcctgg gcctgcactg tcctccctgc tgtcctggac cactcccagc  
 13851 cacttcctgg ggccggggcac gtctgtcagg tctccctggt catggcatcc  
 13901 tccagcctc tgcagtctgt acacactctc ccagcagcat gcctttgccc  
 13951 cagctgtctc ccgtgcctgg gacaccttgc agccacgggc catcacagcc  
 14001 ctgctgggag cttccccaag cccacagtag aatttcttct tgccctcact  
 14051 agagtgggtcc ggagccctag agtctttggg cagttgttgg ggccgacaga  
 14101 gtgaggactc aagtctggcc ctgacttgcg gtgaagggtg gtgaggaggtg  
 14151 gtggggtaag ggcagcctgg ggagccttgg acacagaatt gggggtgata  
 14201 tgggggtcatt cagctggatg tgaccagcac caacgtccca ggggcattcc  
 14251 tggagtaaca gagcccctca ctctggcgcc cactcacctt ggcagcccag  
 14301 cccactcct gaacactctc atgccccttc ttgcagTCGG CCCGCCGCTG  
 14351 CTCACCCTGG TGAACCTTGA AGTGGAAAAT CTGGCGTACA TGCTGGTGTG  
 14401 CATGCAGTGG GCGgtgagtg ggggtgcccc ggaccccggt catacggctg  
 14451 ccgtggcagg aggtgggtgcc tcgggggaca gtacctgcc atgaaggcaa  
 14501 acaggggtgca catgtgctg caacagtgtg gctcacatgt atgctgcaa  
 14551 cagtgtggct cacatgtgtg cgcgcagcag gagagcgagt gtgcccgta  
 14601 ctgtacgtgt ggtggggggg ggttgaggaa cagggggggt gtgggtctct  
 14651 ctcggtgagg gtgtcttccc aggaggagtt gctgggcccga ctctgccagg  
 14701 catctgtgtc cctggcaggg tcttcccca cacaccctgc atgacacctt  
 14751 cgtcactaaa atcagcctcg tgagctggca gggcaaggac cctgttctct  
 14801 tactcagctg agaaaaccag agaggggtgt ggctgtcctt gggctctgag  
 14851 gcaaatcagg cagaagggtt ggatgcctga ggtcctcctc ccaccacca  
 14901 ggctccaga cctccgggca cctggagacc tctcggtatc gcctctgcc  
 14951 tcctctgcag GATTGCTCT GGGCCGCCAG CTTCTATGCC CGCTTCTTCT  
 15001 TATCCTACCT CCCCTTCTAC GCGTCCCTG GGGTGCTGCT CTTCTTTGTT  
 15051 GCTGTCAGgt atggcagga gtggcaggt cacacacagg cgacaggtga  
 15101 cccccactgc agccccccac cagagcttcc ctttcccgt ctgcagaatg  
 15151 gggccagtgg tactgcctcc ctggcttgcg ggtggaatca cataaacaca  
 15201 agcgtggcag gagcccaggg tcggtgggtt tagggagcgt ggcctggctt  
 15251 gtaagtggcc cgggtgggtg cggagctgct ctggactcag cctcacagtg  
 15301 gaactgctc cattcagatt ctttaaacac tggcaagggt gcgatggcca  
 15351 caatcctatt gtacagataa ggaagtcaag gccacttggg gacagctgct  
 15401 ctccagcctc cactcagggt gcctaagtgg tgagctggac ctagggcagt  
 15451 gcccagacct cccacagGG TCCTGGAAAG CCACTGGTTC GTGTGGATCA  
 15501 CACAGATGAA CCACATCCCC AAGGAGATCG GCCACGAGAA GCACCGGGAC  
 15551 TGGGTCAGCT CTCAGgtggg cagcaggggt ggggcccctc ctgggtggg  
 15601 tggggggtcc cagctaggag ccagatggca aagcagggat gaggccctga  
 15651 cggggctgcc aggtggggga tgggtgccgtg gggtcaggga tctgcaacgg  
 15701 cctcctcaca tgtgccccgc cggcttccgg cagCTGGCAG CCACCTGCAA  
 15751 CGTGGAGCCC TCACTTTTCA CCAACTGGTT CAGCGGGCAC CTCAACTTCC  
 15801 AGATCGAGCA CCAgtgagtg tgggtgctgg gggccagtgg gaggtgggga  
 15851 ggggggtcctg ggaggggatc ctgggagggg acccgtgggt ggggcctctc

FIG.2F

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15901 tctggaatct cccacttcag gtgccagcat acgctcccca cccccagCCT  
 15951 CTTCCCCAGG ATGCCGAGAC ACAACTACAG CCGGGTGGCC CCGCTGGTCA  
 16001 AGTCGCTGTG TGCCAAGCAC GGCCTCAGCT ACGAAGTGAA GCCCTTCCTC  
 16051 ACCGCGCTGG TGGACATCGT CAGgtgaggc tgcagcccgg cccctctgtt  
 16101 ctggtggctt cccaggggcc tatgcctacc ctgtgccagg tcagcctcat  
 16151 gctgagcccc cagggtccct gagcctttct gtccacgtcc catgcccttc  
 16201 ctcccttccc cagccttcac gcacacagtg agaatttctg gagcacctac  
 16251 tgcagactca caaacagcag tgcctgcggt gagcaggctt atgcaaacct  
 16301 accccc aaag gctgagggaa aaaagctaac agatccagtt tctcagaagg  
 16351 aaacacttaa cagggactca taaacagaag ccatgtctca gggccgggtg  
 16401 cgggtggctca cgcctgtaat tccagcactt ggggaggctg aggtgggcgg  
 16451 atcacttgag gtcaggagtt cgagaccagc ctggccaaca tggtgaaacc  
 16501 ccgtctctac taaaaaaaaa aaaaaaaaaa aaaacaaaaa aaaaattagc  
 16551 tgggtgtggt ggcagggtgcc cataatccca gctacttggg aggtgagggg  
 16601 aggagaatca cttgaactcg caggggcaga ggttgcagtg agctgagatt  
 16651 gtgcctttgc agtccagcct gggcaacaga gcaagactct ctcaaaaaa  
 16701 aacaaaaaaa ccatgtctca ggcagccaag agttgggaca tcccctcaca  
 16751 cgccctctag aaagaaccct ctatatagca agcttttagg gtgaacccca  
 16801 tgcaggtggt tcttatgaac ctggtgacca ctggagggtta gataagcgct  
 16851 tacaagagga ggttatctat gccatgagct tggcattcag ggtcaagcat  
 16901 cggctcatcag acagttttgc ttgaagatgg cattgccctt gtagcaatgc  
 16951 aggtctctaga gagcttcctg cctccttggg gctgatgttc cttccagcaa  
 17001 aggaacagc aagcaattaa aataacaaat aagtacatta cagaagatgg  
 17051 gcaaaaagaac aatgaaaagc ccctcagggt ggggacaggg gaggggaggg  
 17101 gggcgggccag gcagggggcg cagtttctaa ataggtggta ggggtggcgag  
 17151 tattgacagg ctgacgtgtg agcagggaca gggaggaggg gagaggtctc  
 17201 gccacaggga catctggcaa agagcgttca ggcagagggc acttgaccct  
 17251 gaatgccaaag ctcatggcat agatagccga ggcaggcatg caggcactca  
 17301 gagaagggac acgcccggct tgcacttgg aaagctgccc ctactgggaa  
 17351 tgactggcgg gcaggagtgc aagtggaaaa ggagagcaga ggacactgca  
 17401 gccatccagg cgaggggtga tggggctcag cccttgtggt caccttggag  
 17451 gtggggaaca gagggcagat tccaggctct atacctctgc gcctttgtac  
 17501 acgctgttcc ccttacttgg ttgcccttcc ttcctgtgct ggtgttcaga  
 17551 tgcccaacttc tccctcatga tctctcccag cctgatgtct tgagcccctg  
 17601 ccatttggca cagcccttta gagcgcttgg cacagggtct cctagcagat  
 17651 tgttgacatt tctggctcca ctgcccata tcaggcccaa gatcggtgg  
 17701 gcaggttcca cgtcctctct gtccttgggt tgcagcgccc agcaggaggc  
 17751 agcaatggag aactgggtgc aggagggaca ggcccaccca ggctcatgcc  
 17801 tggacttggc cttggctgcc ctccagctcc cctaccggac acccgtcacc  
 17851 ccggtctaga ttccattcca gagaatgagc attcagctgt tctcccaacc  
 17901 caccctccag cccgcctcgc tgcctgcccc cagggaaggg aaccacagg  
 17951 gaatggggat ctccgctcac acttaccatg ggggatacag ggggtgtagg  
 18001 atcttgcaac tgagctcta acaccaccc cactgcccac cccacctcc  
 18051 cagGTCCCTG AAGAAGTCTG GTGACATCTG GCTGGACGCC TACCTCCATC  
 18101 AGTGAAGGCA ACACCCAGGC GGCAGAGAA GGGCTCAGGG CACGAGCAAC  
 18151 CAAGCCAGCC CCCGGCGGGA TCGATACCCC CACCCCTCCA CTGGCCAGCC  
 18201 TGGGGGTGCC CTGCCTGCCC TCCTGGTACT GTTGTCTTCC CTCGGCCCCC  
 18251 CTCACATGTG TATTCAGCAG CCCTATGGCC TTGGCTCTGG GCCTGATGGG  
 18301 ACAGGGGTAG AGGGAAGGTG AGCATAGCAC ATTTTCCTAG AGCGAGAATT  
 18351 GGGGGAAGC TGTATTATTT ATATTAAAT ACATTACAGAT GTATTATGGA  
 18401 GT

FIG.2G

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1 CTTCGCTTCCCTCGGGGTCTTGCTCGGACCTCGGCCACCGCCTGGGATCC 50

51 CCAGGACTCGTGCGTGCAGCATGGGCGGCGTCCGGGAGCCGGGACCGCGG 100  
1 M G G V G E P G P R 10

101 GAGGGACCCGCGCAGCCGGGGGCACCGCTGCCCACCTTCTGCTGGGAGCA 150  
11 E G P A Q P G A P L P T F C W E Q 27

151 GATCCGCGCGCACGACCAGCCCGGCGACAAGTGGCTGGTCATCGAGCGCC 200  
28 I R A H D Q P G D K W L V I E R R 44

201 GCGTCTACGACATCAGCCGCTGGGCACAGCGGCACCCAGGGGGCAGCCGC 250  
45 V Y D I S R W A Q R H P G G S R 60

251 CTCATCGGCCACCACGGCGCTGAGGACGCCACGGATGCCTTCCGTGCCTT 300  
61 L I G H H G A E D A T D A F R A F 77

301 CCATCAAGATCTCAATTTTGTGCGCAAGTTCCTACAGCCCCTGTTGATTG 350  
78 H Q D L N F V R K F L Q P L L I G 94

351 GAGAGCTGGCTCCGGAAGAACCCAGCCAGGATGGACCCCTGAATGCGCAG 400  
95 E L A P E E P S Q D G P L N A Q 110

401 CTGGTCGAGGACTTCCGAGCCCTGCACCAGGCAGCCGAGGACATGAAGCT 450  
111 L V E D F R A L H Q A A E D M K L 127

451 GTTTGATGCCAGTCCCACCTTCTTTGCTTTCCTACTGGGCCACATCCTGG 500  
128 F D A S P T F F A F L L G H I L A 144

501 CCATGGAGGTGCTGGCCTGGCTCCTTATCTACCTCCTGGGTCCTGGCTGG 550  
145 M E V L A W L L I Y L L G P G W 160

551 GTGCCCAGTGCCCTGGCCGCCTTCATCCTGGCCATCTCTCAGGCTCAGTC 600  
161 V P S A L A A F I L A I S Q A Q S 177

601 CTGGTGTCTGCAGCATGACCTGGGCCATGCCTCCATCTTCAAGAAGTCCT 650  
178 W C L Q H D L G H A S I F K K S W 194

651 GGTGGAACCACGTGGCCCAGAAGTTCGTGATGGGGCAGCTAAAGGGCTTC 700  
195 W N H V A Q K F V M G Q L K G F 210

FIG.3A



FIG.3B

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1401	ATCAGTGAAGGCAACACCCAGGCGGGCAGAGAAGGGCTCAGGGCACCAGC	1450
445	Q	445
1451	AACCAAGCCAGCCCCCGGCGGGATCGATACCCCCACCCTCCACTGGCCA	1500
1501	GCCTGGGGGTGCACTGCCTGCCCTCCTGGTACTGTTGTCCTCCCCCTCGGC	1550
1551	CCCCTCACATGTGTATTTCAGCAGCCCTATGGCCTTGGCTCTGGGCCTGAT	1600
1601	GGGACAGGGGTAGAGGGAAGGTGAGCATAGCACATTTTCCTAGAGCGAGA	1650
1651	ATTGGGGGAAAGCTGTTATTTTTATATTAATAACATTCAGATGTAAAAA	1700

FIG.3C

09806088-01304

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1 GTACAGCGGCAATGGGCGGTGTCGGGGAGCCCGGAGGGGGACTCGGGCCG 50  
1 M G G V G E P G G G L G P 13

51 CGGGAGGGGCCCCGACCGCTGGGGGCGCCCCTACCCATCTTCCGCTGGGA 100  
14 R E G P A P L G A P L P I F R W E 30

101 GCAGATCCGCCAGCATGACCTACCAGGCGACAAGTGGCTGGTCATCGAGC 150  
31 Q I R Q H D L P G D K W L V I E R 47

151 GCCGTGTCTACGACATCAGCCGCTGGGCACAGCGGCACCCAGGGGGTAGC 200  
48 R V Y D I S R W A Q R H P G G S 63

201 CGCATCATCGGCCACCACGG 220  
64 R I I G H H 69

FIG.4

T0210-3303030

13/19

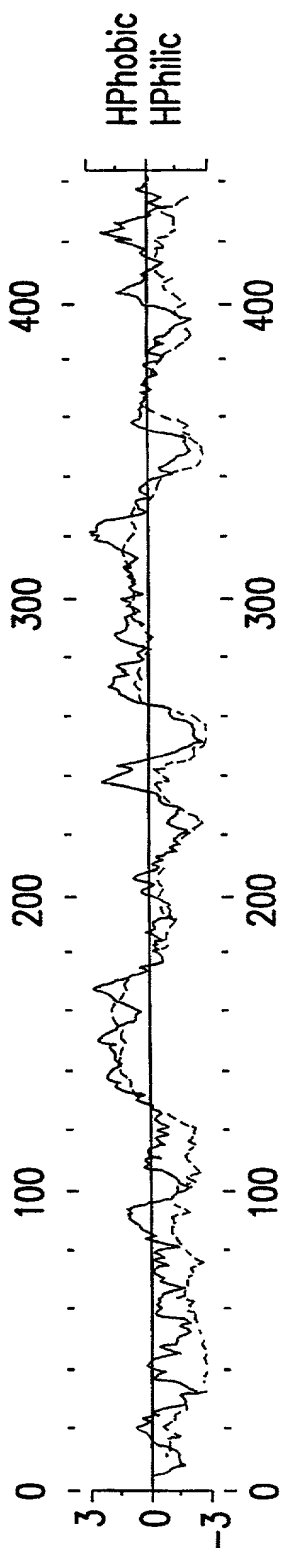
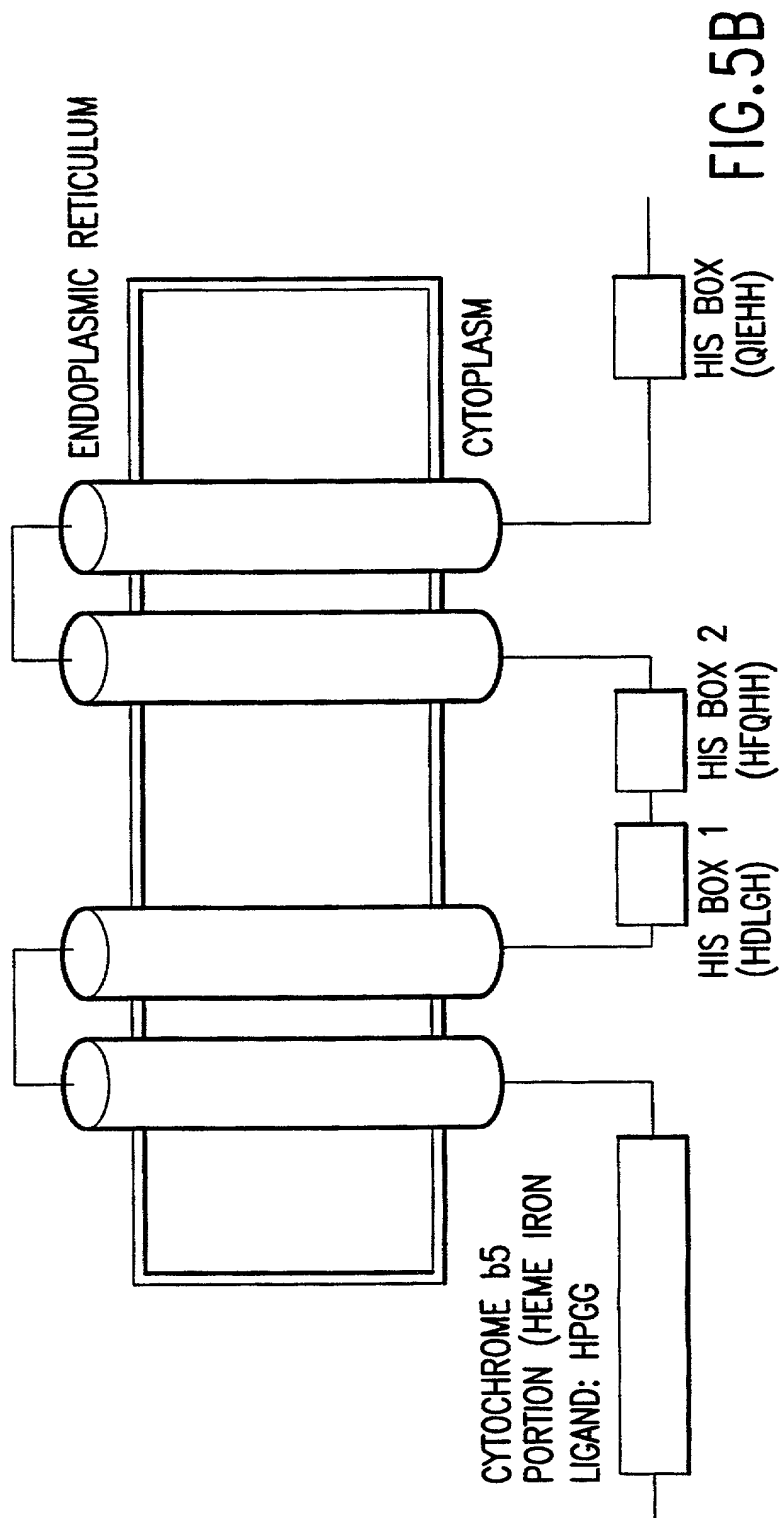


FIG. 5A



14/19

PROFILESCAN of : CYB5rp\_correct\_protein check: 5714 from: 1 to: 445

GETSEQ from bmd, December 2, 1997 14:20.

Compare to profile library: GenRunData:profilesca.n.fil

---

Profile: profiledir:cytochrome\_b5.pr.f

Gap weight: 4.50 Gap Length weight: 0.05

Ave match: 0.27 Ave mismatch : -0.21

(Peptide) PROFILEMAKE v4.40 of: 0191.Msf2{\*} Length: 48

Sequences: 24 MaxScore: 27.58 December 2, 1992 00:07

This profile is derived from PROSITE release 10.0 and has been tested by a database search against SWISS-PROT release 26.0. A comparison of the SWISS-PROT annotation and the results of the database search follows. For further information about this motif, consult the . . .

Profile: profiledir:cytochrome\_b5.pr.f alignment: 1

Quality: 20.77 Gaps: 0

Ratio: 0.43 Length: 48

Normalized quality: 2.91

S 31 HDQPGDKWLVIERRVYDISRWAQRHPGGSRLIGHHGAEDATDAFRAFH 78

|: .: |||. .|||::| . |||. | .||.:||. | ::|

P 1 HNDGEETWLVVNGQVYDITKFLLEHPGGPDVIMEAAGTDATEEFEA1H 48

\*\*\*\*\*  
 \*Cytochrome b5 family, heme-binding domain signature \*  
 \*\*\*\*\*

FIG.6

09/806088-0430

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pir:s68358 hypothetical protein - common sunflower  
Length = 458

Score = 169 (79.4 bits), Expect =  $2.8e-42$ , Sum P(4) =  $2.8e-42$

Identities = 31/85 (36%), Positives = 49/85 (57%)

His box 3

Query: 348 IGHEKHRDWSSQLAATCNVEPSLFTNWFSGHLNFQIEHHLFPRMPRHNYSRVAPLVKSL 407  
+G K +W Q T ++ S + +WF G L FQ+EHHLFPR+PR + ++P+ + L  
Sbjct: 348 VGPPKGDNWFQKTRGTIDACSSWMDWFFGGLQFQLEHHLFPRLPCHLRSISPICREL 407

Query: 408 CAKHGLSYEVKPFALTALVDIVRSK 432  
C K+ L Y F A V +++L+

Sbjct: 408 CKKYNLPYVSLSFYDANVTTLKTLR 432

Score = 133 (62.5 bits), Expect =  $2.8e-42$ , Sum P(4) =  $2.8e-42$

Identities = 21/53 (39%), Positives = 35/53 (66%)

HPGG motif

Query: 26 EQIRAHQPGDKWLVIERRYVDISRWAQRHPGGSRLLIGHGAEDATDAFRAFH 78  
++++ H+ P D W+ I +VY+++ WA+ HPGG + + +D TDAF AFH  
Sbjct: 22 KELKKHNNPNDLWISILGKVYNVTEWAKEHPGGDAPLINLAGQDVTDAFIAFH 74

Score = 118 (55.5 bits), Expect =  $2.8e-42$ , Sum P(4) =  $2.8e-42$

Identities = 25/76 (32%), Positives = 34/76 (44%)

His box 1

His box 2

Query: 165 LAAFILAISSAQSWCLQHDLGHASIFKKSNNHVAQKFMGQLKGFSAHWWNFRHFQHEA 224  
L+ IL ++ Q L HD GH + WN A F+ + G S WW + H HH  
Sbjct: 152 LSGAILGLAWMQIAYLGHADAGHYQMMATRGWNKFAGIFIGNCITGISIAWWKWTNNAHI 211

Query: 225 KPNIFHKDPDVTVPV 240  
N DPD+ P+

Sbjct: 212 ACNSLDYDPLQLHPLM 227

Score = 34 (16.0 bits), Expect =  $2.8e-42$ , Sum P(4) =  $2.8e-42$

Identities = 7/14 (50%), Positives = 9/14 (64%)

FIG. 7A

TOCTO 88090850

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⬆ gp:bou79010 1 PID:g2062403 Borago officinalis delta 6 desaturase mRNA,  
complete cds. (gb:U79010) (NID:2062402)  
Length = 448

Score = 179 (84.1 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42  
Identities = 34/87 (39%), Positives = 48/87 (55%)

His box 3

Query: 348 IGHEKHRDWSSQLAATCNVEPSLFTNWFSGHLNFQIEHHLFPRMPRHNYSRVAPLVKSL 407  
+G K +W Q T ++ + +WF G L FQIEHHLP+MPR N ++P V L  
Sbjct: 338 VGKPKGNNWFEKQTDGTLDISCPPWMDWFHGGLOFQIEHHLPKMPRCNLRKISPYVIEL 397

Query: 408 CAKHGLSYEVKPFLTALVDIVRSLKKS 434  
C K H L Y F A +R+L+ +  
Sbjct: 398 CKKHNLPPYNYASF SKANEMTLRLTLRNT 424

Score = 144 (67.7 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42  
Identities = 23/53 (43%), Positives = 36/53 (67%)

HPGG MOTIF

Query: 26 EQIRAHDPQDKWLVIERRVYDISRWAQRHPGGSRLIGHGAEDATDAFRAFH 78  
++++ HD+PGD W+ I+ + YD+S W + HPGGS + ++ TDAF AFH  
Sbjct: 12 DELKNHDKPGDLWISIQGKAYDVSDWVKDHPGGSFPLKSLAGQEVTDADFVAFH 64

Score = 105 (49.3 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42  
Identities = 22/68 (32%), Positives = 28/68 (41%)

His box 1

His box 2


Query: 176 QSWCLOHDLGHASIFKKSWWNHVAQKFVMGQLKGFSAHWWNFRHFQHHAKPNIFHKDPDV 235  
QS + HD GH + S N F L G S WW + H HH N DPD+  
Sbjct: 153 QSGWIGHDAGHYMMVSDSRLNKFMGIFAANCLSGISGWWKWNHNAHHIACNSLEYDPDL 212

Query: 236 TVAPVFLL 243  
p ++  
Sbjct: 213 QVIPFLV 220

FIG. 7B

FIG. 7B

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 pir:s35157 Delta(6)-desaturase - Synechocystis sp.  
Length = 359

Score = 126 (59.2 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09  
Identities = 21/54 (38%), Positives = 33/54 (61%)

His box 3

Query: 372 F TNWFSGHLNF QIEH LFPMPRHNYSRVAPLVKSLCAKHGLSIEVKPFL TALV 425  
F NMF G LN Q+ H LFP + +Y ++ ++K +C + G+ Y+V P A +  
Sbjct: 292 FWNWFCGLNH QVTH LFPNICH IHYPQLENI IKDVCQEF GVEYKVYPTFKAAI 345

Score = 36 (16.9 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09  
Identities = 6/15 (40%), Positives = 8/15 (53%)

His box 2

Query: 209 GFSAHWWNFR HFQHH 223  
G S+ W +RH H  
Sbjct: 113 GLSSFLWRYR HNHLH 127

FIG.8

09/806088-0101



18/19

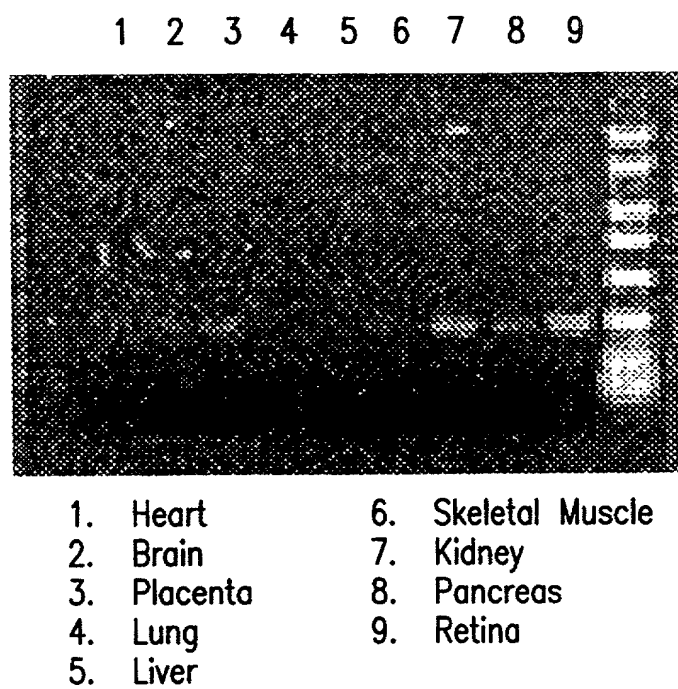


FIG.9A

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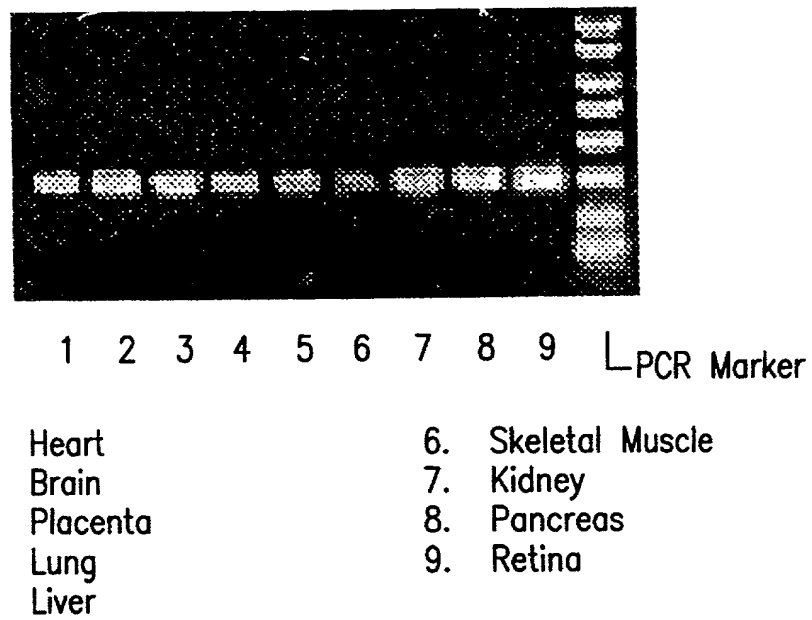


FIG.9B

**DECLARATION AND  
POWER OF ATTORNEY  
FOR UTILITY OR DESIGN  
PATENT APPLICATION  
(37 CFR 1.63)**

☐ Declaration Submitted with Initial Filing  
☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

JUL 13 2001

Attorney Docket Number 20267P

First Named Inventor Petrukhin, et al.

**COMPLETE IF KNOWN**

Application Number 09/806,088

Filing Date March 26, 2001

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DELTA 6 FATTY DESATURASE

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 03/26/2001 as United States Application Number or PCT International

Application Number 09/806,088 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
PCT/US99/23253	PCT	10/09/1999	20267-PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/103,760	10/09/1998	20267PV

**DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application**

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
60/103,760	10/09/1998	
pct/us99/23253	10/05/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number

OR

☒ Registered practitioner(s) name/registration number listed below

Place Customer Number  
Bar Code Label here

Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	Joseph A. Coppola				
Address	Merck & Co., Inc. - Patent Department				
Address	P.O. Box 2000, RY60-30				
City	Rahway	State	NJ	ZIP	07065-0907
Country	USA	Telephone	(732)594-673	Fax	(732)594-4720

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])		Family Name or Surname			
KONSTANTIN		PETRUKHIN			
Inventor's Signature				Date	
Residence: City	Collegeville	State	PA	Country	US
				Citizenship	RU
Post Office Address	Merck & Co., Inc., P.O. Box 2000				
City	Rahway	State	NJ	ZIP	07065-0907

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

## DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)  
Supplemental Sheet

Name of Additional Joint Inventor, if any:

JUL 13 2001

PATENT &amp; TRADEMARK

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

C. THOMAS

CASKEY

Inventor's  
Signature

Date

6/19/01

Residence:  
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Lansdale

State

PA

Country

US

Citizenship

US

Post Office  
Address

Merck &amp; Co., Inc., P.O. Box 2000

City

Rahway

State

NJ

ZIP

07065-0907

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Merck &amp; Co., Inc., P.O. Box 2000

City

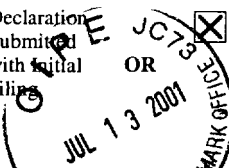
Rahway

State

NJ

ZIP

07065-0907

**DECLARATION AND  
POWER OF ATTORNEY  
FOR UTILITY OR DESIGN  
PATENT APPLICATION  
(37 CFR 1.63)**Declaration  
Submitted  
with Initial  
FilingDeclaration  
Submitted after Initial  
Filing (surcharge  
(37 CFR 1.16 (e))  
required)

Attorney Docket Number

20267P

First Named Inventor

Petrukhin, et al.

**COMPLETE IF KNOWN**

Application Number

09/806,088

Filing Date

March 26, 2001

Group Art Unit

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the specification of which



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				YES	NO
PCT/US99/23253	PCT	10/09/1999	20267-PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>



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☐ Customer Number

OR

☒ Registered practitioner(s) name/registration number listed below

Place Customer Number  
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Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

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Address	Merck & Co., Inc. - Patent Department				
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☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

KONSTANTIN

PETRUKHIN

Inventor's  
Signature

Konstantin Petrukhin

Date

6/01/01

Residence:  
City

Collegeville

State PA

PA

Country US

Citizenship RU

Post Office  
Address

Merck & Co., Inc., P.O. Box 2000

City

Rahway

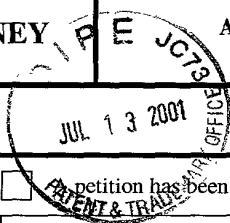
State NJ

ZIP

07065-0907

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

## DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)  
Supplemental Sheet

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])				Family Name or Surname					
C. THOMAS				CASKEY					
Inventor's Signature						Date			
Residence: City		Lansdale		State PA PA		Country US		Citizenship US	
Post Office Address		Merck & Co., Inc., P.O. Box 2000							
City		Rahway		State NJ		ZIP 07065-0907			
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])				Family Name or Surname					
Inventor's Signature						Date			
Residence: City				State		Country		Citizenship	
Post Office Address		Merck & Co., Inc., P.O. Box 2000							
City		Rahway		State NJ		ZIP 07065-0907			
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Given Name (first and middle [if any])				Family Name or Surname					
Inventor's Signature						Date			
Residence: City				State		Country		Citizenship	
Post Office Address		Merck & Co., Inc., P.O. Box 2000							
City		Rahway		State NJ		ZIP 07065-0907			
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])				Family Name or Surname					
Inventor's Signature						Date			
Residence: City				State		Country		Citizenship	
Post Office Address		Merck & Co., Inc., P.O. Box 2000							
City		Rahway		State NJ		ZIP 07065-0907			